SINGLE DOMAIN ANTIBODIES FOR COMPLEMENT REGULATION

TECHNICAL FIELD

[0001] The present invention relates to macromolecules containing single domain antibodies capable of regulating one or more complement pathways by specific binding to human complement factors.

BACKGROUND

[0002] The complement system is part of the innate immune system and plays an important role in protection against invading microorganisms and in maintenance of homoeostasis. Uncontrolled activation or lack of proper regulation of complement is involved in a range of diseases and pharmacological inhibition of complement is believed to represent an attractive strategy to ameliorate disease outcome. This is exemplified by the clinical use of the monoclonal antibody eculizumab which reacts with complement factor C5.

[0003] The complement system is activated by three different proteolytic pathways: The classical pathway (CP), the lectin pathway (LP) and the alternative pathway (AP) (FIG. **1**).

[0004] Therapeutic antibodies used in cancer treatment often rely on activating different parts of the immune system for optimal efficiency. However, only a subset of currently licensed antibodies activates the complement system and complement thus represents a severely underexploited mechanism for clearance of cancer cells but also pathogenic microorganisms.

[0005] A new single domain antibody based technology is therefore provided herein below, which potently and specifically modulate the complement system on target cells.

SUMMARY

[0006] The main object of the present disclosure is to describe single domain antibodies, also termed nanobodies, which are capable of modulating complement activity by specifically targeting epitopes of human complement factors.

[0007] In one aspect, single domain antibodies are provided, which are capable of specifically binding to an epitope of a human complement factor selected from the group consisting of C1q, C3, C4 and/or the proteolytic derivatives C3b and C4b.

[0008] In another aspect, a composition is provided, in particular a pharmaceutical composition, comprising a single domain antibody as defined above.

[0009] In a third aspect, a single domain antibody or a composition as defined above is provided for use as a medicament.

[0010] Another aspect provides a method of modulating the activity of the complement system, said method comprising

[0011] a) providing a composition comprising a human complement factor selected from the group consisting of C1q, C3, C4 and/or the proteolytic derivatives C3b and C4b,

[0012] b) contacting said composition with a single domain antibody as defined above.

[0013] In yet another aspect, a method is provided of treating a disorder associated with complement activation, said method comprising administering a therapeutically effective amount of a single domain antibody or composition as defined above.

[0014] In a further aspect, a method is provided of producing a single domain antibody, said method comprising immunizing a camelid with polypeptide comprising an epitope of a human complement factor selected from the group consisting of the human C1q, C3, C4 and/or the proteolytic derivatives C3b and C4b.

[0015] In a final aspect, a method is provided of detecting the presence of a complement factor selected from the group consisting of the human C1q, C3, C4 and/or the proteolytic derivatives C3b and C4b, wherein a single domain antibody as defined in any of the preceding claims is used as a detection agent.

DESCRIPTION OF DRAWINGS

[0016] FIG. **1**. Activation of the complement system and indications of where the identified Nbs (Nanobodies) inhibit complement.

[0017] FIG. **2**. (A) Binding of C1q to IgG immune complexes in the presence of different Nbs. (B) A subset of these Nbs prevent C4b deposition, and thus CP activation, in a concentration dependent manner. The assay measures C4b deposition in serial dilutions of normal human serum (NHS). (C) Updated comprehensive version of FIG. **2**A. (D) Updated comprehensive version of FIG. **2**B.

[0018] FIG. **3**. (A) Reconstruction based on electron microscopy, of the complex formed between C3b and the Nb D121, asterix marks the position of D121, and the epitope is the C3b C345c domain. (B) The crystal structure of the C-terminal domain of C3b in complex with D121 confirms this epitope mapping. (C) Size exclusion chromatography (SEC) analysis of the binding of C3b to FB in the presence of D121. (D) SDS-PAGE of fractions from SEC analysis of C3b+FB+D121. (E) Updated comprehensive version of FIG. **3**C.

[0019] FIG. **4**. (A) SEC shows that DI62 does not prevent binding of C3b to FB suggesting that it acts by preventing binding of the substrate C3 to both the CP and the AP C3 convertases. Panels B-E compare the ability of DI62 and hC3Nb1 to inhibit the AP (B+C), LP (D) and CP (E). DI62 is able to inhibit both AP, LP and CP. (F) Updated comprehensive version of FIG. **4**A.

[0020] FIG. **5**. (A) SEC analysis of the binding of EWE-hC3Nb1 to C3b. (B) SEC analysis of C3b. (C) SEC analysis of the binding of EWE-hC3Nb1 to C3. (D) SEC analysis of C3. (E) SDS-PAGE analysis of peak fractions from the indicated chromatograms shown in A and C.

[0021] FIG. **6**. (A) SEC analysis of the binding of IgG-Fc-hC3Nb1 to C3b. (B) SEC analysis of IgG-Fc-hC3Nb1. (C) SEC analysis of the binding of IgG-Fc-hC3Nb1 to C3. (D) SEC analysis of C3. (E) SDS-PAGE analysis of peak fractions from the indicated chromatograms shown in A and C.

[0022] FIG. **7**. The inhibitory effects of the nanobodies on AP C3 cleavage. hC3Nb1 (W102A) is a mutant of hC3Nb1 that does not bind to C3 or C3b. (A) Inhibition of C3 deposition using NHS diluted 1:9. (B) Inhibition of erythrocyte lysis using NHS diluted 1:6. The analysis includes measurement of the effect of Nbs on deposition of C3b on a surface of zymosan (A) or measurement of the effect of the Nbs on the lysis of rabbit erythrocytes (B) and is performed in a buffer eliminating the influence of CP and LP.